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Original Research Paper

Isolation of Halotolerant, Thermotolerant and Phosphate Solubilizing Species of *Azotobacter* from the Saline Soil

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ABSTRACT

Soil salinity is a major problem in Maharashtra. Attempt is made to isolate salt-tolerant, thermotolerant, nitrogen fixing, phosphate solubilising *Azotobacter* spp. from the saline soil of Khodashi village in Satara district. Eight *Azotobacter* spp. were isolated from the saline soils. They were confirmed based on morphological, cultural and biochemical characteristics. They were tested for saline and thermal tolerance. The phosphate solubilizing potential of the these *Azotobacter* isolates was qualitatively evaluated by the formation of halos (clear zones) around the colonies growing on solid medium containing tribasic calcium phosphate as a sole phosphorus source. The results showed that phosphate solubilising, salt tolerant and thermotolerant *Azotobacter* spp. could be a promising source for the development of saline-alkali soil-based agriculture.

INTRODUCTION

Soil salinity is one of the most serious environmental problems influencing crop growth around the world. Excessive soil salinity inhibits plant growth by water deficiency and salinity effects (Neumann 1997). Water stress induced by salinity could be regarded as a major factor exerting considerable alterations in plant growth and metabolism (Khan et al. 1994). In drying saline soils, plants are exposed to elevated levels of both water and osmotic stresses because of a simultaneous decrease in matrix and osmotic potential with decreasing soil moisture (Levit 1980, Lovato et al. 1999). The severe salinity induces detrimental effects on plant growth and yield (Abdel-Razek et al. 1991). The salt tolerance is linked through a common mechanism of salt uptake for osmotic adjustment. Salinity affects the growth of the plants by decreasing the availability of water to roots due to the osmotic effect of external salt and exerting toxic effects of excessive salt accumulation within the plant (Munns 1993). The above mentioned effects may directly or indirectly influences physiological processes such as germination, photosynthesis, respiration and metabolite accumulation (Turner & Kramer 1980, Almonsouri et al. 2001).

Azotobacter spp. are most specifically noted for their nitrogen fixing ability but they have also been noted for their ability to produce different growth hormones (IAA and other auxins, such as gibberellins and cytokinins), vitamins and siderophores (Narula et al. 1981, Neito & Frankenberger 1989, Tindale 2000). *Azotobacter* is capable of converting nitrogen to ammonia (Bishop et al. 1980), which in turn is taken up by the plants.

Phosphate solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant phosphorus nutrition. Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of plant growth promoting bacteria is advantageous in the sustainable agricultural practices. Microorganisms tolerating high concentration of salt and yet capable of fixing nitrogen with the additional phosphate solubilizing activities are of importance in increasing saline soil fertility.

Attempt was made to isolate the salt-tolerant, thermotolerant nitrogen fixing *Azotobacter* spp. from saline soil with the potential to solubilize insoluble phosphate that will facilitate the better development of saline-alkali soil-based agriculture.

MATERIALS AND METHODS

Collection of the soil samples and preservation: Soil samples were collected by random sampling procedures from a non-cropped, undisturbed site that was covered by native vegetation from Khodashi village, located 5.3 km distance from Karad city in January 2012. Soil samples were taken

from five different sites from the upper 30 cm of the soil profile, mixed together, kept in a polythene bag, tagged and preserved in refrigerator.

Determination of physico-chemical properties: The soil sample was analysed for its physico-chemical properties. Particle size analysis was done by the pipette method (Gee & Bauder 1986). Soil reaction (pH) and electrical conductivity (EC) were measured in 1:1 soil:water suspension. Organic carbon was determined by the wet oxidation method (Walkley & Black 1934). Available phosphorus and potassium contents in the soils were extracted by Bray's P1 solution and measured on a spectrophotometer and flame photometer, respectively (Bray & Kurtz 1945).

Enrichment of *Azotobacter*: Hundred millilitres of Thompson and Skerman liquid medium (Thompson & Skerman 1979) was used for enrichment. One gram of saline soil sample was transferred into the medium and incubated at 30°C for one week to form a pellicle at surface, which was used to isolate *Azotobacter*.

Isolation of *Azotobacter*: Ashby's Medium – N_2 free mannitol agar was used for isolation of *Azotobacter.* Cultural, morphological and biochemical characteristics of different isolates were studied and confirmed (Bisen & Verma 1996).

Purification: The isolated organisms were purified through repeated plating on Ashby's medium. The purified isolates were then transferred to the slants of nutrient agar medium. One set of these isolates was kept in the polyethylene bags, properly tied and preserved in refrigerator as stock cultures.

Determination of salt tolerance: Nutrient agar slants containing different concentration of sodium chloride (viz. 0%, 0.2%, 0.4%, 0.6%, 0.8% and 1.0%) were inoculated and incubated at 28°C for 48 hours. The growth from different concentrations of NaCl was then compared with the control.

Determination of temperature tolerance: Nutrient agar slants inoculated with isolates were incubated at different temperature (viz. 10°C, 20°C, 30°C, 40°C, 50°C, 60°C).

Screening for phosphate solubilizing activity: Isolates were spot inoculated on Pikovskaya medium (yeast extract-0.5g, dextrose-10g, $Ca_3(PO_4)_2$-10g, $(NH_4)_2SO_4$-0.5g, KCl-0.20g, $MgSO_4.7H_2O$-0.1g, $MnSO_4.H_2O$-0.0001g, $FeSO_4.7H_2O$-0.0001g, agar-18g, distilled water-1000 mL) for detection of their phosphate solubilizing ability and incubated at 37°C for 48 hours. Halo surrounding the colonies was measured and the solubilizing efficiency (SE) was calculated by the following formula:

$$SE = \frac{\text{Solubilization diameter} \times 100}{\text{Growth diameter}}$$

All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Physico-chemical properties of the soil material used were investigated in the laboratory (Table 1). Soil was found to be highly saline and alkaline which is non fertile for most of the crops. Cultural, morphological and biochemical characteristics of all these isolates were studied and confirmed as

Table 1: Physico-chemical properties of the soil material used.

Sr.No.	Parameter	Value
1.	Particle size distribution (%)	Sand = 09, Silt = 26, Clay = 65
2.	pH	8.70
3.	Electrical conductivity (E.C. in mmhos/cm^3)	3.36
4.	Organic carbon (%)	0.35
5.	Available phosphorus (kg/hactare)	15
6.	Available potassium (kg/hactare)	309

Table 2: Salt tolerance of *Azotobacter* isolates.

Azotobacter isolate No.	Salt concentration (%)					
	0.0	0.2	0.4	0.6	0.8	1.0
A-1	++++	++++	+++	++	+	-
A-2	++++	++	+	-	-	-
A-3	++++	++	+	-	-	-
A-4	++++	+++	++	+	+	-
A-5	++++	++++	+++	+++	+	-
A-6	++++	++++	+++	+++	+	-
A-7	++++	++++	+++	++	+	-
A-8	++++	++	+	-	-	-

Azotobacter spp. Salinity test was done for obtaining halotolerant *Azotobacter* isolates (Table 2).

It was found that no isolate survived in 1.0% NaCl concentration. All the isolates showed maximum growth in 0% NaCl while isolates No. 1, 5, 6, 7, 8 showed equal growth both at 0% and 0.2% NaCl concentration. *Azotobacter* isolate No.1, 4, 5, 6 and 7 could tolerate up to 0.8% NaCl concentration whereas 0.4% salt concentration was the tolerable limit for isolate No. 2, 3 and 8.

Temperature tolerance test was done for obtaining the heat tolerant *Azotobacter* isolates (Table 3). All the isolates showed maximum growth at 30°C. Isolates No. 4-7 showed maximum growths both at 30°C and 40°C. No isolate survived at 50°C. Only isolate No. 5 showed growth at 10°C.

Screening of *Azotobacter* isolates for phosphate solubilisation ability: After spot inoculation of isolates on Pikovskaya medium and incubation, growth diameter and solubilisation diameter were measured (Fig. 1) and solubilizing efficiency (SE) was calculated (Table 4). *Azotobacter* isolate A-6 showed highest solubilising efficiency i.e. 178. The order of phosphate solubilization efficiency is: *Azotobacter* isolate A6 > A5 > A4 > A7 > A1 > A2.

CONCLUSION

Eight *Azotobacter* isolates were obtained from saline-alkali soil. All isolates were found to be capable of tolerating 40°C temperature and with the exception of isolate No. 2, 3 and 8 all were capable of tolerating NaCl concentration up to 0.8%. Six out of eight *Azotobacter* spp. isolates showed good phosphate solubilisation abilities. These phosphate solubilizing, salt tolerant *Azotobacter* isolates can be used as suitable substrate for production of biofertilizer for saline-alkali soil-based agriculture.

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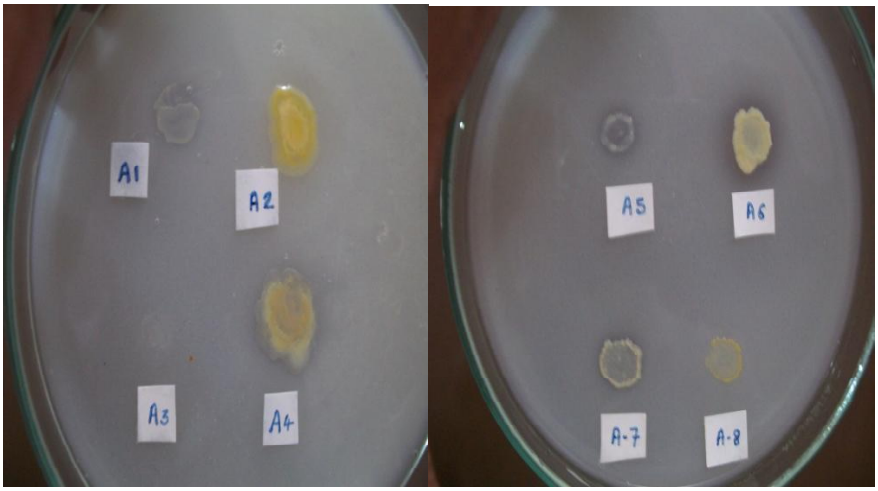

Fig. 1: Screening of *Azotobacter* isolates for inorganic phosphate solubilisation (A1 to A8) on Pikovskaya's medium.

Table 3: Temperature tolerance of *Azotobacter* isolates.

Azotobacter isolate No.	Temperature (°C)					
	10	20	30	40	50	60
A-1	-	+	++++	+	-	-
A-2	-	++	++++	+	-	-
A-3	-	+	++++	+	-	-
A-4	-	++	++++	+++	-	-
A-5	+	++	++++	+++	-	-
A-6	-	++	++++	+++	-	-
A-7	-	++	++++	+++	-	-
A-8	-	+	++++	+	-	-

Table 4: Determination of phosphate solubilising efficiency of *Azotobacter* isolates.

Azotobacter isolate No.	Solubilizing Efficiency (SE)
A-1	138
A-2	120
A-3	-
A-4	167
A-5	171
A-6	178
A-7	140
A-8	-

REFERENCES

Abdel-Razek, A.A., Sadek, S.E. and Naggar, I.M.El. 1991. Evaluation of some maizegenotypes (*Zea mays* L.) with regard to their salt tolerance. Minufia J. Agric.Res., 16: 919-933.

Almonsouri, M., Kinet, J.M. and Lutts, S. 2001.Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil, 231: 243-254.

Bishop, P.E., Jarlenski, D.M.L. and Hetherington, D.R. 1980. Evidence for an alternative nitrogen fixation system in *Azotobacter vinelandii*. In: Proceedings of the National Academy of Sciences, USA. 77: 7342-7346.

Bisen, P.S. and Verma, K. 1996. Handbook ofMicrobiology. CBS Publishers and Distributors, New Delhi.

Bray, R.H. and Kurtz, L.T. 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Science, 59: 39-45.

Gee, G.W. and Bauder, J.W. 1986. Particle-size analysis. In: A. Klute (ed.) Methods of Soil Analysis. Part 1. Agronomy. American Society of America, Madison, WI.

Khan, M.G., Silberbush, M. and Lips, S.H. 1994. Physiological studies on salinity and nitrogen interaction in alfalfa. II. Photosynthesis and transpiration. J. Plant Nutr., 17: 669-682.

Levit, J. 1980. Responses of Plants to Environmental Stresses. Academic Press, New York, London, pp. 365-454.

Lovato, M.B., De-Lemos Filho, J.P. and Martins, P.S. 1999. Growth responses of *Stylosanthes humilis* (Fabaceae) populations to saline stress. Environ Exp. Bot., 41: 145-153.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. Plant Cell Environ., 16: 15-24.

Narula, N., Lakshminarayana, K.L. and Tauro, P. 1981. Ammonia excretion by *Azotobacter chroococcum*. Biotechnology and Bioengineering, 23: 467-470.

Neito, K.F. and Frankenberger, W.T. 1989. Biosynthesis of cytokinins by *Azotobacter chroococcum*. Soil Biology and Biochemistry, 21: 967-972.

Neumann, P. 1997. Salinity resistance and plant growth revisited. Plant Cell Environ., 20: 1193-1198.

Thompson, J.P. and Skerman, V.B.D. 1979. Azotobacteriaceae: The Taxonomy and Ecology of the Aerobic Nitrogen Fixing Bacteria, Academic Presss, London.

Tindale, A.E., Mehrotra, M., Ottem, D. and Page, W.J. 2000. Dual regulation of catercholate siderophore biosynthesis in *Azotobacter vinelandii* by iron and oxidative stress. Microbiology, 146: 1617-1626.

Turner, N.C. and Kramer, P.J. 1980. Adaptation of Plants to Water and High Temperature Stress. John Wiley and Sons, New York.

Walkley, A. and Black, I.A. 1934. An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci., 63: 251-263.